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Single quantum dots are increasingly used for single-molecule imaging, thanks to their brightness, functionalization versatility and relatively small size. So far, however, experimentalists have limited themselves to standard analysis methods developed for single-dye imaging, which are notoriously short-lived, and require statistical analysis of many trajectories. We show that there is much more information to be extracted from long single quantum dot trajectories than afforded by these standard methods. In particular, we show by simulations and using real data obtained in live cells that it is possible to identify the presence of several diffusion modes within a single trajectory and characterize these different diffusion modes quantitatively. Our approach is based on the probability distribution of square displacements measured for different time lags. We use both simulated data and live cell single quantum dot tracking data to illustrate the capability of our method and compare it to other commonly used techniques. This method was recently used to demonstrate the existence of two diffusion regimes for GPI-anchored proteins in the membrane of HeLa cells, identified respectively with diffusion in and out of raft domains (1).

Reference:
1. Pinaud et al., Traffic 10 (2009) 691.

1058-Plat

Far Field Fluorescence Super Resolution Imaging of Molecular Scale Biological Structures

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We have recently demonstrated measurements of relative distances between individual, spectrally distinct fluorescent probes that are attached on a biological molecule with precision and accuracy <1nm. Here we extend this technique to measure relative distances between two (or more) fluorophores of the same spectral characteristics.

We show that the improved resolution afforded by our microscope imaging system allows characterizing biological structures at the molecular scale. In one application we applied our technique to image individual dimers of Endothelial cadherin (E-cadherin) molecules. At 1mM Ca++ we find the majority of the dimers in an extended configuration, consistent with the crystal structure of the full C-cadherin ectodomain. Interestingly, we observe a separate population in a less extended configuration, possibly related to flexibility of the E-cadherin binding interface. As a function of the free Ca++ concentration we observe a continuous, cooperative transition of the extension of the molecules from the rigid extended configuration to a collapsed state. The apparent Kd=70uM and degree of cooperativity ~1.5 from our measurements are consistent with various previous indirect investigations (e.g. intrinsic fluorescence, proteolytic sensitivity, circular dichroism, NMR etc).

These results clearly demonstrate the power of our approach to image individual molecular scale structures and detect conformational changes at the nanometer scale, establishing it as a unique Structural Biology tool, that can operating in ambient, physiological conditions and is based exclusively on optical far-field fluorescence imaging.

Platform O: Membrane Structure I

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Molecular Organization of Cholesterol in Phospholipids

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Cholesterol-enriched domains in cell membranes are involved in a wide variety of cellular activities including protein sorting, signal transduction, and host-pathogen interactions. Several models describing the structural organization of cholesterol-phospholipid membranes have been proposed, but consensus on cholesterol-lipid organization within the cell membrane has not yet been reached. For instance, the "superlattice" model suggests regular distribution of cholesterol in a hexagonal lipid matrix, whilst the condensed complex model states that cholesterol and lipids form complexes only at a particular stoichiometry. Moreover, current models constrain cholesterol placement to exclusively being within the lipid acyl chains regardless of its mole fraction (χ_{CHOL}). Here we present a systematic structural study of cholesterol-dipalmitoylphosphatidylcholine (DPPC) mixed monolayers at the air-liquid interface by surface pressure-molecular area Langmuir isotherms, epifluorescence microscopy, X-ray reflectivity (XR) and grazing incident-angle X-ray diffraction (GIXD) using synchrotron radiation. The electron density profile across the monolayer, derived from XR data, demonstrates that the vertical position of cholesterol relative to phospholipids strongly depends on χ_{CHOL}. At a range of sterol concentrations, cholesterol and DPPC form new alloy-like mixed domains of short-range order and stoichiometry defined by the overall molecular composition of the film. Since these data cannot be explained within the existing models, we propose a new model of cholesterol-lipid organization in mixed monolayers that is consistent with both the "condensing" effect of cholesterol and a sharp increase in its chemical activity at $\chi_{CHOL}>0.4$. Our data above all does not support the theory that membrane domains exist as independent ordered lipid/cholesterol entities ready to accommodate protein molecules.

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In-situ Measurement of Cholesterol Transport in Model-Membrane Systems Studied by Time Resolve Small Angle Neutron Scattering and Comparison with MD Simulation

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Cholesterol is essential for a myriad of biological functions, but its excess is toxic. Cholesterol levels are maintained by various cholesterol metabolic pathways which depend critically on its intracellular transport and homeostasis. Any disorder in intracellular cholesterol distribution will lead to diseases, from neurodegenerative, such as Niemann Pick TYPE-C and Alzheimer, to cholelithiasis and atherosclerosis. Unfortunately, the understanding of intracellular transport of cholesterol has lagged behind other aspect of cholesterol metabolism due to limitations in the techniques used to date. These limitations have resulted in reported cholesterol transport rates showing huge inconsistencies. This is due to the fact that these measurements are not done in-situ, but rather require biochemical isolation of cholesterol-donor and cholesterol-acceptor vesicles. Another important limitation has been the need for a biochemical tag on cholesterol which has a significant effect on the resulting rates of transfer. This work presents in-situ Time-Resolved Small Angle Neutron Scattering (in-situ TR-SANS)studies of the Intra- and Inter-membrane cholesterol exchange rates in POPC model vesicles. This technique does not require any biochemical tag and transport of cholesterol from donor to acceptor vesicles can be measured continuously by following the changes in the scattering intensity. Interestingly our approach finds that trans-membrane flipping rates of cholesterol are much slower without any foreign particles in contrast to high flipping rates reported in literature. Molecular dynamics simulations have also been performed to investigate the energetic and kinetic behavior of cholesterol. We found that simulation results are in agreement with our SANS results, providing a more detailed thermodynamic description at the molecular level. Such a synergistic approach combining TR-SANS and MD simulation will provide new insight into the ongoing efforts of understanding cholesterol traffic and related disorders.

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Sterol Transfer from Vesicles to MBCD is Governed by the Extent of Sterol Superlattice

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Cholesterol transfer regulates the intracellular distribution and the metabolism of cholesterol, thus having a direct impact on cholesterol homeostasis in cells. This work investigates the effect of lipid lateral organization on sterol transfer from liposomes to methyl-B-cyclodextrin (MBCD), a water-soluble macrocyclic compound able to deplete sterols from membranes. Large unilamellar vesicles (LUVs) composed of POPC, dehydroergosterol (DHE) and Dansyl-PE were examined. DHE content was varied in steps of 0.4 mol% in a range of concentrations covering the theoretically predicted critical mole fractions (C_r,e.g., 20.0, 22.2, 25.0, 33.3, 40.0 and 50.0 mol%) for maximal sterol superlattice formation. The molar ratio of DHE to Dansyl-PE was kept constant (15:1) in all samples. The rate of sterol transfer was monitored based on the fluorescence resonance energy transfer (FRET) between DHE (donor) and Dansyl-PE (acceptor). When DHE is transferred from LUVs to MBCD, FRET efficiency is decreased and, consequently, the fluorescence intensity of Dansyl-PE is decreased over time. The initial rate of the DHE transfer was found to vary with DHE content in the original membrane in a biphasic manner, reaching a local maximum at all C_r examined. This result demonstrates that the rate of DHE transfer from LUVs to MBCD is governed by the extent of sterol superlattice in the liposomal membrane, a conclusion different from that given in a previous study[1]. Both studies showed the same general trend, i.e., that sterol chemical potential increases with increasing sterol content. The difference lies in the fine details of how sterol transfer rate varies with sterol content in the immediate vicinity of C_r (AHA, DOD and PDOH).

[1] Ali *et al.* (2007) PNAS 104:5372-7.